

Accepted Manuscript

Title: Improved surface plasmon resonance biosensing using silanized optical fibers

Author: Iulia Arghir Dragana Spasic Bert E. Verlinden Filip Delpoort Jeroen Lammertyn



PII: S0925-4005(15)00521-3
DOI: <http://dx.doi.org/doi:10.1016/j.snb.2015.04.069>
Reference: SNB 18374

To appear in: *Sensors and Actuators B*

Received date: 7-1-2015
Revised date: 19-4-2015
Accepted date: 22-4-2015

Please cite this article as: I. Arghir, D. Spasic, B.E. Verlinden, F. Delpoort, J. Lammertyn, Improved surface plasmon resonance biosensing using silanized optical fibers, *Sensors and Actuators B: Chemical* (2015), <http://dx.doi.org/10.1016/j.snb.2015.04.069>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

- A robust FO-SPR biosensor was developed by improving the gold layer adhesion
- The gold layer adhesion was dramatically enhanced by introducing a silane compound
- The silanized FO showed stability under mechanical, chemical and thermal stress
- The improved gold layer stability allowed repeated bioassay implementation

Improved surface plasmon resonance biosensing using silanized optical fibers

Iulia Arghir¹, Dragana Spasic¹, Bert E. Verlinden², Filip Delport¹ and Jeroen Lammertyn^{1*}

¹KU Leuven, Department of Biosystems, MeBioS – Biosensor group, Willem de Croylaan 42, B-3000 Leuven, Belgium

²Flanders Centre of Postharvest Technology, Willem de Croylaan 42, B-3000 Leuven, Belgium

*Author to whom correspondence should be addressed:

E-mail: jeroen.lammertyn@biw.kuleuven.be

Phone: +32 (0) 16 32 1459; Fax: +32 (0) 16 32 2955

Abstract

Coupling surface plasmon resonance (SPR) to optical fiber (FO) technology has brought tremendous advancements in the field by offering attractive advantages over the traditional prism-based SPR platforms, such as simplicity, cost-effectiveness and miniaturization. However, the performance of the existing FO-SPR sensors widely depends on the adhesion of the gold (Au) layer to the FO silica core, thereby often representing a major limiting factor in achieving the properties of the benchmark SPR systems. In this paper, we used (3-mercaptopropyl)trimethoxysilane (MPTMS) as an adhesion promoter for developing robust Au surfaces on the three-dimensional (3D) FO-SPR sensing probe. Carefully prepared FO substrates were first silanized using a wet chemistry approach, with MPTMS concentrations ranging from 2.5 to 24 mM, and subsequently exposed to a drying treatment at room temperature (RT) or at 100°C, before coating them with a ~50 nm Au plasmonic film. Differently prepared silanized FOs were next used for evaluating their sensitivities, by performing refractive index (RI) measurements in sucrose dilutions. Advanced statistical analysis of the obtained data indicated that using 8 mM MPTMS solution coupled with a RT post-drying treatment is an efficient way of producing FOs with dramatically improved Au adhesion properties. The role of the MPTMS underlayer was further investigated by exposing the reference and silanized FOs to stress conditions, such as strong mechanical (adhesion tape tests), chemical (piranha solution treatments) and thermal variations. Although additional studies using scanning electron microscopy (SEM) revealed changes in the Au film morphology after all these endurance tests, the silanized FOs exhibited an enhanced robustness while retaining the overall sensor's capabilities. In contrast, the reference FOs consistently failed the mechanical and chemical tests, while only resisting under thermal variations. Moreover, the improved resistance of the silanized FO-SPR probes allowed them to be reused up to three times with no significant loss in the sensor performance, while implementing bioassays based on two types of bioreceptors (a DNA aptamer against thrombin protein and a polyclonal antibody against human Immunoglobulin E – hIgE). All these results might represent a step forward in the fabrication of more robust and reusable FO-SPR biosensors, featuring great potential for developing highly-sensitive biochemical assays.

Keywords: Fiber optic – surface plasmon resonance (FO-SPR); (3-mercaptopropyl)trimethoxysilane (MPTMS) coupling agent; Improved gold (Au) adhesion; Stability and robustness enhancements; Antibody- and aptamer-based bioassays; Reusable biosensor.

Abbreviations: FO-SPR, Fiber Optic – Surface Plasmon Resonance; RI, Refractive Index; NPs, Nanoparticles; APTMS, [(3-aminopropyl)trimethoxysilane]; MPTMS, [(3-mercaptopropyl)trimethoxysilane]; hIgE, human Immunoglobulin E; MES, 2-(N-morpholino)ethanesulfonic acid; NHS, N-hydroxysulfosuccinimide; EDC, 1-ethyl-3-[dimethylaminopropyl]carbodiimide; SAM, Self-Assembling Monolayer; DIW, Deionized Water; PBS, Phosphate Buffer Saline; TGK, (3-hydroxyamino)methane-glycine-potassium; ANOVA, analysis of variance; RT, room temperature.

1. Introduction

The surface plasmon resonance (SPR) technology is a well-established detection and analysis tool with many proven applications in health care diagnostics [1], biotechnology and life-science research [2], the agro-food sector, security and environmental monitoring [3]. SPR sensors exploit the mono- or polychromatic polarized light, which excites the metal (typically gold – Au or silver – Ag) / dielectric (e.g. target sample – usually a liquid) interface to generate propagating plasmonic waves, highly sensitive to the refractive index (RI) changes in the sample [4,5]. These changes can be used to measure real-time interactions between proteins, lipids, nucleic acids or even low molecular weight molecules such as drugs [6], providing thus valuable information on target quantification and biomolecular kinetics. Despite all this potential, SPR devices that are already available on the market (e.g. Biacore [7,8]) are still rarely used outside the research centers because most of them are expensive bulky systems due to the associated complex optical equipment, precision mechanical components and sophisticated pump-driven microfluidic schemes [9,10]. In addition, although the SPR platform has been promoted as a promising tool capable of handling label-free bioassays [11], it still poses a number of challenges in developing sensitive and specific bioassays [12].

Mostly for portability purpose, Jorgenson and Yee [13] proposed in 1993 a novel SPR system based on a Au – coated fiber optic (FO) design. Numerous similar FO-SPR devices have been introduced since then [14-16] in an attempt to enhance the performance of the entire sensing platform and make it comparable with the traditional SPRs [17]. In this context, it has been shown that by accurately controlling the chemical interactions at the sensor surface [18], by implementing labeling approaches using Au or magnetic nanoparticles (NPs) [19], by patterning the active sensing area using well-controlled nanostructuring techniques [20], and/or just by simply improving the adhesion of the Au thin layer deposited on the glass substrate [21], the specificity, sensitivity and other features of the sensor can be further improved.

In our previous research it has been demonstrated that the in-house developed FO-SPR platform [22,23] has great potential for applications outside the specialized research environment due to the low-cost components, its capacity for multichannel measurements in screening applications and the possibility for improved sensitivity through the incorporation of NPs into bioassays, a feature not feasible with the classical microfluidics – based SPR systems [24]. Moreover, this sensing platform was successfully used both, for protein- [25] and DNA- [26] based bioassays, making it useful for different applications. However, the performance of the existing FO-SPR sensors mostly depends on the adhesion of the Au layer sputtered on the FO silica core within the sensitive zone, thereby often representing the major limiting factor in achieving the expected properties. Most commonly, a good adhesion can be achieved by introducing an intermediate metallic ultra-thin (several nm) layer of Cr or Ti

[27]. However, these compounds may absorb part of the propagated light during the SPR excitation, altering further the sensor's signal response and thus lowering its sensitivity [28,29]. Moreover, the deposition of such extremely thin metallic underlayers with good evenness onto non-planar FO substrates requires specialized, complex and expensive equipment.

Recently, several groups reported the use of organosilanes as efficient attachment layers for either Au NPs [30] or films [31] on flat glass substrates. These organic compounds can be homogeneously deposited resulting in thin films with no additional light absorption, thus with minimal plasmon damping effects [32]. Two silane types are widely used for such purpose: (i) an amino functional silane [(3-aminopropyl)trimethoxysilane – APTMS] [33] and (ii) a thiol functional compound [(3-mercaptopropyl)trimethoxysilane – MPTMS] [34]. Although both compounds combine good chemical and physical properties with excellent transparency, the APTMS is a less preferred candidate for this purpose due to the weak electrostatic NH_2 – Au interactions [35] compared to the strong covalent S – Au bonds formed by the MPTMS [36,37]. Such an approach might be an effective way to attract various Au architectures (e.g. NPs [37] or nanorods – NRs [38]) directly on the FO silica core.

In this paper, the MPTMS was used as an adhesion promoter for preparing robust and consistent Au thin films onto the three-dimensional (3D) surface of the in-house developed FO-SPR sensor [22,23]. We demonstrated that the presence of the MPTMS layer enhances the stability and robustness of the sensor under mechanical, chemical and thermal stress conditions. The robustness was further evaluated by implementing two different bioassays on the FO substrate: (i) an immunoassay for human Immunoglobulin E (hIgE) [39] detection and (ii) an aptamer based bioassay for α -thrombin [40] detection. The silanized FOs featured a surface capable of resisting complete regeneration with piranha solution (a mixture of sulfuric acid and hydrogen peroxide) up to three times while retaining the overall bioassay performance and sensitivity. This research represents a step forward in the fabrication of robust and reusable FO-SPR biosensing platforms suitable for implementing highly-sensitive bioassays.

2. Materials and Methods

2.1. Reagents. The chemicals in this work were of high quality analytical grade (99.99% purity, unless otherwise specified). Acetone, sodium hydroxide (NaOH), sulfuric acid (97% H_2SO_4) and acetic acid were purchased from Chem-Lab, Belgium. Hydrogen peroxide (30% H_2O_2), anhydrous toluene, ethanol, methanol, sodium acetate, ethanolamine, MPTMS, 2-(N-morpholino)ethanesulfonic acid [41], bovine serum albumin (98% BSA), trizma base, streptavidin and anti-hIgE (ϵ -chain specific) polyclonal antibody were supplied by Sigma-Aldrich, Belgium. Glycine, D(+)-sucrose, sodium chloride (NaCl), 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC) and N-hydroxysulfosuccinimide (Sulfo-NHS) were acquired from Fischer Scientific, Belgium. Biotinylated self-assembling monolayer (SAM) and carboxylic acid-SAM were obtained from GERBU Biotechnik GmbH, Germany. Tween 20 BioChemica and potassium phosphate dibasic (K_2HPO_4) were purchased from Applichem, Germany. Human α -thrombin was supplied by Bio-Connect, the Netherlands. The biotinylated anti-thrombin DNA aptamer [42] was purchased from Integrated DNA Technologies, Belgium. Myeloma hIgE kappa was obtained from Athens Research and Technology, USA. Au NPs (20 nm diameter, concentration of 7×10^{11} NPs/mL) were purchased from BBI international (Cardiff, United Kingdom). All dilutions were prepared with deionized water (DIW) purified by a Milli-Q 50 system (Millipore, USA). A nitrogen (N_2) gas stream with an estimated volumetric flow rate of $35 \text{ m}^3/\text{h}$ was used to dry the samples

during the different processing steps. A phosphate buffer saline (PBS) pH 7.4 was prepared by dissolving a 10 g PBS packet (Neogen Corporation, UK) in 1 L of DIW. The TGK buffer (pH 8.3) was obtained by preparing an aqueous solution containing 25 mM trizma base, 192 mM glycine and 5 mM K_2HPO_4 .

2.2. Fabrication and silanization of the FO-SPR sensors. Optical probes were manufactured by cutting a TEQS multimode FO (Thorlabs, Germany) with a diameter of 400 μm in pieces with a consistent length of 3.6 cm. A SPR – sensitive zone of 0.6 cm was constructed at one side by mechanically removing the FO jacket and dissolving the polymer cladding in acetone, followed by drying the FO with dust free tissues. The hydroxyl groups (OH^-) on the FO surface were activated by immersing the samples in piranha solution (3:7 % v:v H_2O_2 : H_2SO_4) for 15 min. The FOs were subsequently rinsed with DIW and methanol, and afterwards dried under N_2 gas flow. The prepared probes were then subjected to a silanization process, using a wet chemistry approach. Briefly, the samples were ultrasonicated for 2 h in anhydrous toluene, containing 0.3 M acetic acid and MPTMS. The silane concentrations tested for optimizing the protocol were: 0, 2.5, 8, 16 and 24 mM. The acetic acid was used to favor the hydrolysis reaction while minimizing the condensation inside the solution. Next, the silanized probes were cleaned in methanol and dried using a N_2 gas stream. Additionally, half of the samples were in a subsequent step exposed to 5 min drying treatment at 100°C and compared to those kept at room temperature (RT). In the end, the silanized probes were coated by a thin Au layer (~ 50 nm) using a sputter coater (Quorum Q150T ES, UK). The DC plasma was activated for 7 min at 21 mA in Ar atmosphere kept at $2.5 \cdot 10^{-2}$ mbar, while the FOs were installed on a rotating stage (100 rpm) for improving the Au FO coverage during the sputtering process. Reference FOs were directly coated by Au without any prior silanization step. At least five FO-SPR probes per specified condition (n) were prepared and tested for optimizing the silanization protocol.

2.3. FO-SPR setup and RI measurements. The role of the MPTMS layer in promoting Au adhesion to FO silica core was systematically investigated using various methods. Firstly, the adhesion improvement of each FO was challenged by rolling it over an adhesive tape (with ~ 5 FO revolutions in 10 sec). Secondly, the influence of silane layer on the FO sensitivity was tested by RI measurements in serial sucrose dilutions (0, 2.5, 4, 6, 7.5, 9, 11, 13 % w/w). The Brix values of the prepared sucrose dilutions (see the corresponding RI values in the supplementary data, Table S1) were checked with a digital refractometer (Atago Palette PR-32, Japan). The interchangeable FO probes were typically inserted into adequate SMA connectors (Avantes, USA) and mounted on a computer-controlled SPR measuring platform (for details see supplementary data, Figure S1). The FOs were kept one minute in each sucrose solution during the RI measurements, unless otherwise specified. Depending on the applied silanization treatment, five to nine FOs were prepared. The obtained SPR wavelength shifts were further analyzed using a mixed analysis of variance (ANOVA) model, performed in SAS (v. 9.3, USA). By definition the SPR shift of the 0 % sucrose solution was put equal to zero. This was taken into account by forcing the regression lines for all FOs through the point with RI of 0 % sucrose and a SPR shift of zero. The slope represents the sensitivity of the FO and quantifies the RI effect. To quantify the fabrication treatment effect a separate slope was estimated for each fabrication treatment level. The fabrication treatment factor had nine levels being the four silanization levels combined with or without an oven treatment at 100°C and the non-treated (reference) control level. The FO was taken as a random factor grouping the data resulting from the same FO accounting for their correlation structure. In addition, multiple comparisons were implemented in conjunction with the Bonferroni algorithm to identify the statistically significant treatments [43].

The overall robustness of the FO tips was next tested by performing similar RI measurements in sucrose dilutions (0, 2, 4, 8, 12 % w/w) on both reference and silanized FOs, before and after successively immersing them for 30 sec in piranha solution. Piranha solution was selected for its well-known capability of removing organic molecules from substrates [29,44], allowing thus an effective and complete surface regeneration (see 3.3). Additional experiments for testing the robustness of the FO tips were performed by exposing the FOs to thermal variations in DIW. To simulate polymerase chain reaction (PCR), which is frequently implemented on this platform [45], 40 cycles from 50 to 98 °C, with a total duration of 100 min were performed using a standard PCR thermocycler (Px2 Thermal Cycler, Thermo Electron Corporation, USA).

2.4. Morphological characterization of FO-SPR surfaces. A field-emission scanning electron microscope (FE-SEM, JEOL 7401F) was used to observe the changes in the Au film morphology of the samples exposed to the above-mentioned mechanical, chemical and thermal treatments. The FO Au – coated area was first carefully diced using a blade, then installed on the SEM holder using a conducting carbon paste in order to minimize the charging effects, and afterwards inserted into the SEM vacuum chamber. The specimens were specifically mounted to allow side, top or tilted observations. A low accelerating voltage (2 kV) was used to reduce the charging effects and to extract more information close to the sample surface.

2.5. Bioassay implementation on the FO-SPR probes. The reusability of the silanized FO-SPR probes was tested by implementing two different biorecognition layers on the FO surface and consecutively removing them with piranha solution (30 sec). In this context, two bioassays were used as model systems: (i) detection of thrombin with a biotinylated DNA aptamer and (ii) detection of hIgE with an anti-IgE polyclonal antibody. The FO sensor tips were incubated overnight at 4°C, with 0.1 mM biotin- or carboxyl-SAM before implementing the aptamer- or antibody – based bioassays, respectively. After incubation, the FO sensor tips were washed in ethanol and kept in MES buffer (50 mM, pH 6) until further use. For immobilizing the aptamer, the FOs were first immersed in 5 µM streptavidin in MES buffer (20 min), followed by incubation with 500 nM of thrombin specific biotinylated DNA aptamer solution in PBS buffer containing 300 nM NaCl (20 min). The functionalized FOs were used to detect five different concentrations of human α -thrombin (0, 34, 68, 135 and 270 nM) in TGK buffer containing 0.15% BSA and 0.1% Tween 20. For antibody immobilization on the FO-SPR tips, the carboxylic groups of SAM were first activated for 15 min with 0.4 M EDC and 0.1 M sulfo-NHS in a 50 mM MES buffer (pH 6). Further, the FOs were immersed for 30 min in 10 mM sodium acetate buffer (pH 5.5) with 20 µg/mL of anti-hIgE antibody. After blocking with 50 mM ethanolamine in PBS, the sensor was used to detect 10 µg/mL hIgE in PBS buffer containing 0.01% Tween 20. Here, Au NPs functionalized with anti-hIgE polyclonal antibody were applied as a secondary label to amplify the SPR signal. Au NPs (pH 9) were incubated with 1 mg/mL anti-hIgE antibody for 20 min on a rotator. Next, 750 µL of 0.5% BSA was added per 1 mL of Au NPs-anti hIgE antibody solution, incubated for 1 h on the rotator, afterwards centrifuged at 7000 rpm for 20 min in PBS with 0.01% Tween 20 buffer, and finally stored at 4°C. In both bioassays, FO-SPR sensor tips were washed prior each step using a buffer containing 1 M NaCl and 50 mM NaOH for removing nonspecific bound molecules.

3. Results and Discussion

3.1. Optimization of MPTMS deposition protocol on the FO-SPR sensor tips

The silanization protocol was optimized to deposit the MPTMS silane layer on the FO-SPR sensor tips with the aim to improve the Au layer adhesion without compromising the FO-SPR sensitivity. Different MPTMS deposition parameters were applied (for details, see 2.2), resulting in nine distinct experimental conditions, each tested using five to nine FO sensor tips. The SPR wavelength shift was recorded in real-time as a function of RI change for each sucrose solution (Figures 1A-C). For each combination of MPTMS concentration and curing temperature, the results were collected and statistically analyzed (using the mixed ANOVA model) to determine whether different protocols lead to significant differences in sensitivities (represented as bar graphs in Figure 1D). The reference FOs exhibited an absolute sensitivity of 1723 nm/RIU, whereas the silanized FOs had a sensitivity range between 1625 and 1931 nm/RIU depending on the MPTMS concentration and the corresponding drying condition. As it can be seen in Figure 1D, certain treatments (indicated with different letters, e.g. A, AB, B and C) indeed resulted in statistically different sensitivities between the FOs (with the significance level, $p < 0.05$). FOs kept at RT after the MPTMS deposition process (columns labeled as I) did not show statistically significant changes in their sensitivity compared to the reference FOs (the column labeled as R), except those treated with 8 mM silane that exhibited a significant improvement (indicated with C). Similarly, the silanized FOs exposed to 100°C treatment (columns labeled as II) resembled mostly the reference samples, except for a statistically significant decrease in the sensitivity performance for the 2.5 mM silane condition. According to these results, the presence of the MPTMS intermediate layer generally maintained the sensitivity of the sensors compared to the reference FOs since most of the bar graphs fall within the B or AB group, with only two applied protocols significantly influencing the sensitivity (A and C groups). The observed decrease in performance (group A) can be attributed to the combination of low MPTMS concentration and additional heating step at 100°C. In contrast, the noticeable improvement in sensitivity (group C) is most likely due to the superior Au film adhesion on the FO surface (as it will be further shown in this manuscript). Such an observation is also strengthened by other studies showing that a good MPTMS self-assembly occurs when the solution concentration is kept below 10 mM. At higher concentrations, the MPTMS quality might be affected by the competition between the self-polymerization and the surface dehydration, which both depend on the trace quantity of water in the solvent and on the silica substrate [46].

Next, the influence of the different silanization strategies on the Au adhesion to the FO surface was tested. This was assessed by rolling the FOs over an adhesive tape (with a constant velocity of ~0.5 revolutions/sec) and estimating the amount of Au transferred to the tape by its visual inspection. In this study, a decisive “YES/NO” threshold was applied, where “NO” represented perfect adhesion (e.g. no visually noticeable Au transferred to the tape after the test). The percentage of the samples with unaffected Au layer after the applied test is shown in Figure 2A. As can be noticed, most of the silanized FOs sustained this test substantially better compared to the reference FOs, which showed complete removal of the Au layer. These results clearly confirmed the overall improvement of Au resistance to mechanical stress conditions in the presence of the MPTMS layer. Although some variability was observed for different silanization protocols, the number of samples prepared with 8 mM MPTMS solution at RT that persisted the tape test was superior compared to other silanization conditions. This was in line with the previous results that showed enhanced sensitivity of FO-SPR sensors prepared following this silanization protocol (see Figure 1D).

Following the tape test, the FO-SPR sensors were further exposed to RI measurements in serial sucrose dilutions, similarly to the previous experiment. The differences in sensitivity obtained before and after the tape test are shown in Figure 2B. Surprisingly, the majority of

the silanized FOs were found to exhibit superior sensitivity after performing the Au tape tests. Although further experiments are needed to prove the hypothesis, we can already speculate that the observed feature might be attributed to the changes in the Au film structure when exposed to the adhesive tape surface, which enables the formation of small Au-free “islands” on the FO, possibly creating a microstructured Au surface previously reported to enhance the sensitivity [47]. Moreover, a similar correlation between changes in the Au film structure and increased sensitivity was observed after exposing the silanized FOs to the chemical treatment and is addressed further in the manuscript.

In conclusion, the presented results indicate that using 8 mM MPTMS solution without exposing the FOs to high temperature treatment is an efficient way for producing silane intermediate layer that improves the Au adhesion while maintaining the overall sensor's stability. Therefore, these conditions were further applied in the subsequent experiments for producing silanized FO-SPR sensing probes.

3.2. Evaluation of the FO-SPR sensor robustness

The silanized FO-SPR probes, fabricated according to the optimized silanization protocol (see 3.1), were subsequently used for evaluating the robustness of the sensor under chemical conditions. In this context, RI measurements were carried out in sucrose dilutions using reference and silanized FOs before and after consecutive immersions in piranha solution, until no measurable SPR signal response was obtained (see supplementary data, Figure S2). Figure 3 shows the change in sensitivity over different cycles of piranha treatment both, for reference (gray) and silanized (red) FOs. The raw experimental data on which Figure 3 was based, are presented in Figure S3 from the supplementary data. As noticed, the reference FO could be re-used only once for RI measurements, while the silanized FOs showed an SPR response for 8 successive rounds of piranha treatment. This could be explained by the superior chemical resistance of the Au film in the presence of the MPTMS layer.

Surprisingly, the silanized FO-SPR sensors were also exhibiting a consistent increase in the sensitivity values during the piranha immersion steps. The observed behavior was explained by the noticeable changes in the Au film morphology when imaging the FOs surface with the SEM (Figure 4). The Au layer was almost completely absent for the reference FO after the second piranha immersion (Figures 4A and B), whereas the silanized FOs were exposed 8 times to piranha solution before observing major damages to the Au film, and consequently no SPR response (Figures 4C-F). Moreover, due to the improved adhesion properties, the Au film only gradually degraded during piranha immersion cycles enabling the formation of localized 3D Au ripples on the FO surface (Figures 4D-F). These newly formed structures locally increased the active sensing area, possibly causing the sensitivity shifts observed in Figure 3.

The robustness of the FO-SPR sensors was further tested using temperature variations as described in 2.3. Figure 5A shows the sensitivity calculated by performing RI measurements on both reference (gray) and silanized (red) FOs, before and after the thermocycling procedure (the raw data are shown in the supplementary data, Figure S4). In contrast to the results obtained when exposing the FOs to piranha, the repeated temperature cycles did not lead to major differences in sensitivity between reference and silanized FOs. However, a soft deterioration of the Au film was observed in the SEM micrographs (Figures 5B and C). This resembled the “nanoporous-like” semi-continuous Au layers previously reported to cause strong plasmonic behaviors due to the presence of “hot spots” that formed among the small gaps in the metallic film under the applied thermal stress [41,48,49]. Although such structures might be expected to cause similar plasmonic phenomena on the FO-SPR sensor, their absence could be explained with the milder exposure conditions used in the experiments with

the FO-SPR sensor (e.g. temperature cycling between 50 and 98°C compared to the higher temperature of 200°C typically reported in literature). Therefore, the observed morphological changes in the Au layer could be only the onset of the entire process that can be further induced by more closely mimicking the reported temperature conditions. Although these experiments might give an interesting insight into the behavior of our FO-SPR platform and its sensing capacity, they are not within the scope of this manuscript and will not be addressed here.

3.3. Evaluation of the FO-SPR sensor reusability

The presented results so far pointed out that the MPTMS – assisted Au sputtering process is an effective way to produce FO-SPR sensors with superior endurance and stability due to the improved quality of the resulted Au film. In order to evaluate further the robustness of the sensor and its capability to be reused, reference and silanized FOs were prepared as described in 3.1 and subsequently used to implement bioassays with two different bioreceptors – a thrombin aptamer and an antibody against hIgE. The biomolecular recognition layers were successively removed in piranha solution, followed by their reimplementation on the FO substrates (as detailed in 2.5).

3.3.1. Aptamer-based bioassay

Streptavidin coated reference and silanized FOs were functionalized with biotinylated anti-thrombin DNA aptamer and used to detect five concentrations of thrombin (0-270 nM range) in a TGG buffer. Figure 6A shows a typical FO-SPR sensorgram obtained for the protein detection using the DNA-based bioreceptor (represented here only for the silanized FO). Based on the obtained sensorgrams, the linear calibration curves for both, reference and silanized FOs were calculated, exhibiting a similar behavior (Figure 6B). Afterwards, the FOs were thoroughly washed in piranha for 30 sec followed by re-settling the entire bioassay. This was repeated until no SPR signal response was observed (see supplementary data, Figure S5). The bar graphs in Figure 6C represent the SPR wavelength shift of the FOs (for 270 nM thrombin) for each of the implemented bioassays. As can be noticed, the reference FO could be used only once due to the extensive damage of the Au film, which is in line with the previous results (see Figures 4A and B). In contrast, the MPTMS-coated samples could be reused up to three times without a major loss in the sensing performance. To control whether the biorecognition layer has been completely removed each time, the FO-SPR probes were used for detecting the highest concentration of the thrombin immediately after piranha treatment. An example is shown in Figure 6D, where no shift was obtained, proving the complete surface regeneration. The absence of nonspecific interaction between thrombin protein and the Au – coated sensor surface is shown in Figure S6 from the supplementary data.

3.3.2. Antibody-based bioassay

Next, a bioassay with an antibody as bioreceptor was implemented on both reference and silanized FOs, similar to the aptamer-based bioassay. Anti-hIgE antibody was immobilized on the sensor surface through EDC/NHS chemistry, followed by the detection of hIgE and signal amplification step using Au NPs functionalized with polyclonal anti-hIgE antibody [37,50] (Figure 7A). In a similar way (as described in 3.3.1), the FOs were then subjected to

successive cycles of piranha treatments (for 30 sec) and bioassay reimplementation until no SPR signal was finally obtained (see supplementary data, Figure S7). Figure 7B shows the SPR wavelength shift of both reference and silanized FOs for each of the implemented bioassays. The reference FOs provided no SPR response after the first piranha immersion (due to the Au film delamination), while the MPTMS-coated samples could be reused for up to three times, in a similar way as described for the aptamer-based assay. Nonspecific interaction between hIgE or anti-hIgE antibody with the Au sensor surface was not detected in this experimental setting (see Figure S8 from the supplementary data).

4. Conclusions

In this paper, we reported an improved fabrication protocol for the FO-SPR sensing platform, based on the introduction of an intermediate MPTMS organic compound, which proved to enhance the adhesion and quality of the thin Au layer, subsequently sputtered onto the curved FO substrate. The MPTMS deposition protocol was experimentally optimized and statistically validated. The superior quality of the Au film in the presence of the MPTMS layer was systematically investigated by exposing the sensors to mechanical, chemical and thermal stress conditions. The reference FOs consistently failed the mechanical and chemical harsh exposures, while only resisting under soft thermal variations. In contrast, the silanized FOs definitely exhibited an enhanced robustness under all these tests, demonstrating also a good sensing capacity. Additional SEM imaging of the sensor's surface exposed to piranha treatment showed changes in the Au film morphology of the silanized FOs, possibly causing sensitivity enhancements. The overall improved resistance of the silanized FO-SPR probes allowed them to be reused up to three times with no significant loss in the sensor performance, when reimplementing both an aptamer- and an antibody – based bioassays. These results represent a step forward in the fabrication of robust and reusable FO-SPR biosensors, featuring a great potential for developing highly-sensitive biochemical assays. The proposed fabrication methodology contributes also to the low-cost feature of the sensing platform by reducing the Au usage (due to the reusability of this FO-SPR biosensor), and thus by possibly providing a better strategy in the global preservation of the noble metals.

Supplementary data

Supplementary data associated with this article is available and includes: (1) a complete description of the FO-SPR setup used for carrying out the optical measurements; (2) the table giving the correlation between the sucrose dilutions and the associated refractive index units (RIU); (3) raw data plots used for preparing the bar graphs shown in Figures 3 and 5; (4) results from the reimplementation cycles and nonspecific binding tests for both bioassays.

Acknowledgements

The research leading to these results has received funding from: the European Commission's Seventh Framework Programme (FP7/2007-2013) under the grant agreement BIOMAX (project n° 264737), the KU Leuven Industrial Research Fund (NanoDiag) and the Flemish Institute for the Promotion of Innovation through Science and Development (IWT Sensors for Food VIS-trajectory and IWT Innovation mandate of F.D.). The authors kindly thank Pieter

Baatsen and Analytical Electron Microscopy – KU Leuven Core Facility from “Center of Human Genetics – Molecular and Developmental Genetics” for the SEM access.

References

- [1] Y. Chen, H. Ming, Review of surface plasmon resonance and localized surface plasmon resonance sensor, *Photonic Sens* 2 (2012) 37-49.
- [2] M. Piliarik, H. Vaisocherová, J. Homola, Surface Plasmon Resonance Biosensing, in: A. Rasooly, K. Herold (Eds.), *Biosensors and Biodetection*, Humana Press 2009, pp. 65-88.
- [3] X. Guo, Surface plasmon resonance based biosensor technique: a review, *Journal of biophotonics* 5 (2012) 483-501.
- [4] I. Abdulhalim, M. Zourob, A. Lakhtakia, Surface Plasmon Resonance for Biosensing: A Mini-Review, *Electromagnetics* 28 (2008) 214-242.
- [5] D.R. Shankaran, K.V. Gobi, N. Miura, Recent advancements in surface plasmon resonance immunosensors for detection of small molecules of biomedical, food and environmental interest, *Sensors and Actuators B: Chemical* 121 (2007) 158-177.
- [6] Y. Yuan, Y. Benshun, X. Jinsheng, L. ZhaoHui, Surface Plasmon Resonance Biosensors and its Application, *Bioinformatics and Biomedical Engineering, 2007 ICBBE 2007 The 1st International Conference on 2007*, pp. 1043-1046.
- [7] S.C.B. Gopinath, Biosensing applications of surface plasmon resonance-based Biacore technology, *Sensors and Actuators B: Chemical* 150 (2010) 722-733.
- [8] L. Jason-Moller, M. Murphy, J. Bruno, Overview of Biacore systems and their applications, *Current protocols in protein science / editorial board, John E Coligan [et al]* Chapter 19 (2006) Unit 19.13.
- [9] E. Kretschmann, The Determination of the Optical Constants of Metals by Excitation of Surface Plasmons, *Z Physik* 241 (1971) 313-324.
- [10] A. Otto, Excitation of nonradiative surface plasma waves in silver by the method of frustrated total reflection, *Z Physik* 216 (1968) 398-410.
- [11] A.D. Taylor, J. Ladd, J. Homola, S. Jiang, Surface Plasmon Resonance (SPR) Sensors for the Detection of Bacterial Pathogens, in: M. Zourob, S. Elwary, A. Turner (Eds.), *Principles of Bacterial Detection: Biosensors, Recognition Receptors and Microsystems*, Springer New York 2008, pp. 83-108.
- [12] B. Nguyen, F.A. Tanious, W.D. Wilson, Biosensor-surface plasmon resonance: Quantitative analysis of small molecule–nucleic acid interactions, *Methods* 42 (2007) 150-161.
- [13] R.C. Jorgenson, S.S. Yee, A fiber-optic chemical sensor based on surface plasmon resonance, *Sensors and Actuators B: Chemical* 12 (1993) 213-220.
- [14] B.D. Gupta, R.K. Verma, Surface Plasmon Resonance-Based Fiber Optic Sensors: Principle, Probe Designs, and Some Applications, *Journal of Sensors* 2009 (2009) 12.
- [15] J.F. Masson, Y.C. Kim, L.A. Obando, W. Peng, K.S. Booksh, Fiber-optic surface plasmon resonance sensors in the near-infrared spectral region, *Applied spectroscopy* 60 (2006) 1241-1246.

- [16] M. Mitsushio, M. Higo, Simplification and evaluation of a gold-deposited SPR optical fiber sensor, *Analytical sciences : the international journal of the Japan Society for Analytical Chemistry* 20 (2004) 689-694.
- [17] I. Arghir, F. Delport, D. Spasic, J. Lammertyn, Smart design of fiber optic surfaces for improved plasmonic biosensing, *New Biotechnology* (2015), doi: 10.1016/j.nbt.2015.1003.1012.
- [18] D.R. Shankaran, N. Miura, Trends in interfacial design for surface plasmon resonance based immunoassays, *Journal of Physics D: Applied Physics* 40 (2007) 7187.
- [19] L. Wang, T. Li, Y. Du, C. Chen, B. Li, M. Zhou, et al., Au NPs-enhanced surface plasmon resonance for sensitive detection of mercury(II) ions, *Biosensors and Bioelectronics* 25 (2010) 2622-2626.
- [20] J.N. Anker, W.P. Hall, O. Lyandres, N.C. Shah, J. Zhao, R.P. Van Duyne, Biosensing with plasmonic nanosensors, *Nat Mater* 7 (2008) 442-453.
- [21] W.M. Kim, S.H. Kim, K.S. Lee, T.S. Lee, I.H. Kim, Titanium nitride thin film as an adhesion layer for surface plasmon resonance sensor chips, *Applied Surface Science* 261 (2012) 749-752.
- [22] K. Knez, K.P. Janssen, J. Pollet, D. Spasic, J. Lammertyn, Fiber-optic high-resolution genetic screening using gold-labeled gene probes, *Small (Weinheim an der Bergstrasse, Germany)* 8 (2012) 868-872.
- [23] J. Pollet, F. Delport, K.P.F. Janssen, K. Jans, G. Maes, H. Pfeiffer, et al., Fiber optic SPR biosensing of DNA hybridization and DNA-protein interactions, *Biosensors and Bioelectronics* 25 (2009) 864-869.
- [24] J. Homola, Present and future of surface plasmon resonance biosensors, *Analytical and Bioanalytical Chemistry* 377 (2003) 528-539.
- [25] D.T. Tran, K. Knez, K.P. Janssen, J. Pollet, D. Spasic, J. Lammertyn, Selection of aptamers against Ara h 1 protein for FO-SPR biosensing of peanut allergens in food matrices, *Biosensors and Bioelectronics* 43 (2013) 245-251.
- [26] K. Knez, K.P. Janssen, D. Spasic, P. Declerck, L. Vanysacker, C. Denis, et al., Spherical nucleic acid enhanced FO-SPR DNA melting for detection of mutations in *Legionella pneumophila*, *Analytical chemistry* 85 (2013) 1734-1742.
- [27] B.A. Sexton, B.N. Feltis, T.J. Davis, Characterisation of gold surface plasmon resonance sensor substrates, *Sensors and Actuators A: Physical* 141 (2008) 471-475.
- [28] M.B. Ali, F. Bessueille, J.M. Chovelon, A. Abdelghani, N. Jaffrezic-Renault, M.A. Maaref, et al., Use of ultra-thin organic silane films for the improvement of gold adhesion to the silicon dioxide wafers for (bio)sensor applications, *Materials Science and Engineering: C* 28 (2008) 628-632.
- [29] I. Doron-Mor, Z. Barkay, N. Filip-Granit, A. Vaskevich, I. Rubinstein, Ultrathin Gold Island Films on Silanized Glass. Morphology and Optical Properties, *Chemistry of Materials* 16 (2004) 3476-3483.
- [30] Y. Jin, X. Kang, Y. Song, B. Zhang, G. Cheng, S. Dong, Controlled nucleation and growth of surface-confined gold nanoparticles on a (3-aminopropyl)trimethoxysilane-modified glass slide: a strategy for SPR substrates, *Analytical chemistry* 73 (2001) 2843-2849.
- [31] A.K. Mahapatro, A. Scott, A. Manning, D.B. Janes, Gold surface with sub-nm roughness realized by evaporation on a molecular adhesion monolayer, *Applied Physics Letters* 88 (2006) 151917.
- [32] T.G. Habteyes, S. Dhuey, E. Wood, D. Gargas, S. Cabrini, P.J. Schuck, et al., Metallic adhesion layer induced plasmon damping and molecular linker as a nondamping alternative, *ACS nano* 6 (2012) 5702-5709.

- [33] K. Fujiwara, H. Kasaya, N. Ogawa, Gold nanoparticle monolayer formation on a chemically modified glass surface, *Analytical sciences : the international journal of the Japan Society for Analytical Chemistry* 25 (2009) 241-248.
- [34] M. Lamy de la Chapelle, H. Shen, N. Guillot, B. Frémaux, B. Guelorget, T. Toury, New Gold Nanoparticles Adhesion Process Opening the Way of Improved and Highly Sensitive Plasmonics Technologies, *Plasmonics* 8 (2013) 411-415.
- [35] S. Yochelis, E. Katzir, Y. Kalcheim, V. Gutkin, O. Millo, Y. Paltiel, Formation of Au-Silane Bonds, *Journal of Nanotechnology* 2012 (2012) 8.
- [36] S. Zeng, X. Yu, W.-C. Law, Y. Zhang, R. Hu, X.-Q. Dinh, et al., Size dependence of Au NP-enhanced surface plasmon resonance based on differential phase measurement, *Sensors and Actuators B: Chemical* 176 (2013) 1128-1133.
- [37] Q. Zhang, C. Xue, Y. Yuan, J. Lee, D. Sun, J. Xiong, Fiber Surface Modification Technology for Fiber-Optic Localized Surface Plasmon Resonance Biosensors, *Sensors* 12 (2012) 2729-2741.
- [38] J. Cao, M.H. Tu, T. Sun, K.T.V. Grattan, Wavelength-based localized surface plasmon resonance optical fiber biosensor, *Sensors and Actuators B: Chemical* 181 (2013) 611-619.
- [39] S. Kim, J. Lee, S.J. Lee, H.J. Lee, Ultra-sensitive detection of IgE using biofunctionalized nanoparticle-enhanced SPR, *Talanta* 81 (2010) 1755-1759.
- [40] M. Famulok, Turning aptamers into anticoagulants, *Nat Biotech* 22 (2004) 1373-1374.
- [41] P. Pavaskar, I.-K. Hsu, J. Theiss, W. Hsuan Hung, S.B. Cronin, A microscopic study of strongly plasmonic Au and Ag island thin films, *Journal of Applied Physics* 113 (2013) 034302.
- [42] K. Janssen, K. Knez, D. Spasic, J. Lammertyn, Nucleic Acids for Ultra-Sensitive Protein Detection, *Sensors* 13 (2013) 1353-1384.
- [43] J.M. Bland, D.G. Altman, Multiple significance tests: the Bonferroni method, *BMJ* 310 (1995) 170.
- [44] Y. Lin, Y. Zou, R.G. Lindquist, A reflection-based localized surface plasmon resonance fiber-optic probe for biochemical sensing, *Biomed Opt Express* 2 (2011) 478-484.
- [45] J. Pollet, K.P. Janssen, K. Knez, J. Lammertyn, Real-time monitoring of solid-phase PCR using fiber-optic SPR, *Small (Weinheim an der Bergstrasse, Germany)* 7 (2011) 1003-1006.
- [46] M. Hu, S. Noda, T. Okubo, Y. Yamaguchi, H. Komiyama, Structure and morphology of self-assembled 3-mercaptopropyltrimethoxysilane layers on silicon oxide, *Applied Surface Science* 181 (2001) 307-316.
- [47] T. Karakouz, D. Holder, M. Goomanovsky, A. Vaskevich, I. Rubinstein, Morphology and Refractive Index Sensitivity of Gold Island Films, *Chemistry of Materials* 21 (2009) 5875-5885.
- [48] U.K. Chettiar, P. Nyga, M.D. Thoreson, A.V. Kildishev, V.P. Drachev, V.M. Shalaev, FDTD modeling of realistic semicontinuous metal films, *Appl Phys B* 100 (2010) 159-168.
- [49] R.P. Van Duyne, J.C. Hulteen, D.A. Treichel, Atomic force microscopy and

surface-enhanced Raman spectroscopy. I. Ag island films and Ag film over polymer

nanosphere surfaces supported on glass, *The Journal of Chemical Physics* 99 (1993) 2101-2115.

- [50] X. Hong, E.A.H. Hall, Contribution of gold nanoparticles to the signal amplification in surface plasmon resonance, *Analyst* 137 (2012) 4712-4719.

Figure Captions

Figure 1. The influence of the silanization treatment on the FO-SPR sensitivity. Typical sensor's calibration curves measured in serial sucrose dilutions for: reference FOs (A) and FOs silanized with different MPTMS concentrations ranging from 2.5 to 24 mM, (B) kept at room temperature (RT) or (C) exposed to high temperature treatment (100°C). The RI units correspond to a range of sucrose concentrations between 0 and 13% w/w (see Table S1 in the supplementary data). The slopes of the displayed curves represent the average sensitivity of the FOs for a specific treatment. (D) Bar graphs summarizing the averaged sensitivity values per treatment, as obtained from the mixed statistical ANOVA model. The error bars represent the standard errors ($n = 5$ to 9 samples, depending on the applied silanization treatment), subsequently used in the Bonferroni algorithm to determine the statistically significant differences between the silanization conditions (e.g. the threshold letters A, AB, B and C).

Figure 2. The influence of the different silanization conditions on the Au layer adhesion. (A) Bar graphs representing the percentage of the FOs unaffected by the tape test when prepared following different protocols for MPTMS deposition. The error bars on the graphs were calculated using a binomial distribution algorithm ($n = 5$ to 9 samples, depending on the applied silanization treatment). (B) Corresponding bar graphs with calculated relative difference in the sensitivity before and after the adhesive tape tests. The error bars represent the standard error ($n = 3$ to 8 samples, depending on the number of the unaffected/measurable FOs after the tape test).

Figure 3. SPR sensitivity obtained in different sucrose dilutions for a reference (gray) and a silanized (red) FO-SPR probe, before and after piranha immersions (raw data can be found in Figure S3 of the supplementary data). The x-axis represents the number of piranha immersion cycles. The error bars indicate the standard deviation ($n = 5$ samples).

Figure 4. SEM images demonstrating the improved adhesion of the silanized FOs. (A, B) SEM micrographs showing the reference FO after the second piranha immersion. The arrows indicate small Au pieces remaining on the FO surface. (C-F) SEM images displaying the silanized FO surface after the 2nd (C, D), 4th (E) and 8th (F) piranha treatments, respectively. The arrows indicate 3D Au ripples gradually formed on the FO surface with each piranha

immersion cycle. The insets of (A) and (B) represent corresponding low magnification SEM images of the FOs before dipping them in piranha solution.

Figure 5. (A) SPR sensitivity obtained in different sucrose dilutions for a reference (gray) and a silanized (red) FO-SPR probe, before and after the thermocycling procedure (raw data can be found in Figure S4 of the supplementary data). The error bars show the standard deviation ($n = 5$ samples). (B, C) Corresponding SEM micrographs showing the reference and the silanized FOs, respectively, after the thermocycling step. The circular markers indicate Au nanoporous regions formed during the thermal variations.

Figure 6. FO reusability for the aptamer – based bioassay. (A) Typical sensorgram showing different bioassay stages (block A – streptavidin immobilization, block B – the biotinylated DNA coupling to the FO-SPR sensor and block C – binding of the thrombin to the aptamer). For simplicity, only the sensorgram corresponding to the silanized FO was represented. (B) Corresponding linear calibration curves acquired after 20 min detection of each thrombin concentration with both, reference and silanized FOs. The error bars correspond to the standard deviation ($n = 3$ samples). (C) SPR wavelength shifts obtained for the detection of 270 nM thrombin with reference (gray) and silanized (red) FOs for each of the implemented bioassays. (D) Thrombin binding (270 nM) to the sensor surface after the piranha treatment.

Figure 7. FO reusability for the antibody – based bioassay. (A) Typical sensorgram showing different steps of antibody – based bioassay implementation (carboxylic SAM activation using EDC/NHS chemistry, anti-hIgE antibody immobilization, the hIgE detection followed by an amplification step with Au NPs functionalized with anti-hIgE antibody). (B) SPR wavelength shift measured for a reference (gray) and a silanized (red) FO for each of the implemented bioassays. The error bars correspond to the standard deviation ($n = 3$ samples).

Ms. Iulia Arghir obtained a BSc diploma (2009) in Medical Physics and an MSc degree (2011) in Materials Science from the Faculty of Physics, University of Bucharest, Romania. Her master thesis research was mostly focused on the fabrication and characterization of advanced materials and nanostructures for optoelectronic devices. She next joined the Biosensor group within the division of Mechatronics, Biostatistics and Sensors (MeBioS) of the Biosystems Department at KU Leuven, Belgium as a PhD fellow. Her current research is in the framework of plasmonic biosensing, with particular focus on development and improvement of fiber optic – surface plasmon resonance (FO-SPR) biosensors.

Dr. Dragana Spasic received her MSc and PhD degrees in Medical Sciences from KU Leuven, Belgium in 2004 and 2009, respectively. She was a post-doctoral researcher at the Faculty of Medicine (KU Leuven, 2009 – 2011). Currently she is a postdoc in the Biosensor group at the Faculty of Bioscience Engineering (KU Leuven). She is involved in research and projects related to development of bioassays (e.g. aptamer selection and application as bioreceptors, biofunctionalization of nanomaterials) and biosensors, particularly FO-SPR sensors, towards diagnostic tools with food and medical applications.

Dr. Filip Delpont holds an MSc in Bioscience Engineering (2006, KU Leuven, Belgium), as well as a PhD in Bioscience Engineering (2012, KU Leuven, Belgium). Additionally, a post graduate in corporate finance was obtained in 2013. Starting in 2012 he worked as a postdoctoral researcher for Flanders Food, Sensors for Food. In 2013 he received an Innovation mandate grant to valorize the FO-SPR sensor concept which is currently continued by externally attracted funding. He is involved in research and projects related to development of bioassays (e.g. surface chemistry, biofunctionalization of nanomaterials) and biosensors (signal analysis, software methodology), particularly FO-SPR sensors.

Prof. Dr. Jeroen Lammertyn holds an MSc in Bioscience Engineering (KU Leuven, Belgium) and in Biostatistics (UHasselt, Belgium), as well as a PhD in Bioscience Engineering (2001, KU Leuven, Belgium). From 2002 he worked as a postdoctoral researcher and spent one year at Pennsylvania State University, USA. Since 2005 he is Professor in the division of Mechatronics, Biostatistics and Sensors (MeBioS) of the Biosystems department (KU Leuven, Belgium). His main research interests involve biosensors development, nanohybrid materials, microfluidics and bioassays development. He is author or co-author of 100+ peer reviewed research papers, and acts as reviewer for many international journals.













